Tissue engineering and the development of Apligraf, a human skin equivalent

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ABSTRACT

In recent years, skin grafting has evolved from the initial autograft and allograft preparations to biosynthetic and tissue-engineered living skin replacements. This review details the pioneering work of numerous investigators that led to the following precursors of tissue-engineered skin replacement: cultured autologous keratinocyte grafts, cultured allogeneic keratinocyte grafts, autologous/allogeneic composites, acellular collagen matrices, and cellular matrices. It also discusses the rationale for the development of the newer products and describes the technical advances leading to the development of Apligraf(R), a tissue-engineered human skin product. Key words: wounds, skin equivalent, tissue engineering, Apligraf.

INTRODUCTION

The first recorded use of skin grafting occurred around 1000 BC in the Orient, where full-thickness skin grafts (epidermis and entire dermal layer) were applied to wounds in a primitive type of cosmetic surgery.1 As skin grafting techniques improved, their use moved westward. Autogeneic split-thickness grafts (epidermis and partial dermal layer) were introduced in Europe in the late 1800s.

Skin grafts may compensate for tissue loss at multiple levels by acting as an occlusive dressing, as a skin replacement, and as a stimulus for healing.2 The most common reason for grafting is to treat burns. Other situations in which skin grafting is beneficial include the treatment of venous and pressure ulcers, skin repair after removal of cutaneous malignancies, and skin repair after excision of certain birthmarks (eg, giant hairy nevi).

Full- or split-thickness autografts are usually readily available, can be taken from a variety of donor sites, have low metabolic requirements, and cover the wound permanently. Full-thickness grafts reduce wound contraction. Blister formation, susceptibility to shear forces, and chronic inflammation are occasional problems with split-- thickness grafts.

Human skin allografts are also useful, particularly in burn patients, in whom donor sites may be limited. Fresh skin grafts vascularize more readily than do frozen ones. Allogeneic cadaveric skin, whether fresh, frozen, or lyophilized, is commonly available. Lyophilized allografts have a long shelf life, but the barrier function of the epidermal layer is lost in processing. It is generally believed that all allografts are eventually rejected. Natural suppression of the immune system in burn patients temporarily inhibits rejection of cadaveric skin, but immunosuppressant drugs are sometimes required to prolong allograft life.3

Cadaveric skin and amnionic membranes are used for temporary wound cover. These membranes adhere well, vascularize quickly, and may supply growth factors to the wound. However, amnionic membranes have high water permeability, need frequent replacement, and require time to procure.4 The most serious concern with allogeneic skin is the possibility of transmitting infection, particularly the human immunodeficiency virus (HIV) or hepatitis. Even with rigorous screening, it is still possible that skin could be harvested from an HIV-infected but seronegative donor.

Other useful alternatives have been investigated. Porcine xenografts act much like human cadaveric skin. The disadvantages include limited shelf life and possible bacterial contamination.5 Like human allografts, porcine xenografts are eventually rejected, making them a temporary rather than permanent wound covering. Bovine collagen, available in strips and sheets or as a film or powder, is immediately available and easy to handle. It adheres well and results in excellent vascularity. Disadvantages are its lack of barrier function and elasticity and its inability to prevent bacterial invasion.6 Eventually, a split-thickness graft is required in patients who receive bovine collagen xenografts.

Numerous biologic and synthetic dressings or temporary skin substitutes are available, including membranes, gels, foams, and spray-on materials. These dressings serve as barriers until wounds heal or other graft material becomes available.5 Many of these products are not ideal for treating large wounds. They differ from the tissue-engineered skin products discussed below in that they do not contain living cells and do not perform like human skin.7

RATIONALE FOR THE DEVELOPMENT OF A HUMAN SKIN EQUIVALENT

Because of the high morbidity and mortality among patients who lose a large amount of skin, skin grafts serve a vital function. However, there are difficulties involved. Often the donor does not have enough skin for autografting. Repeated harvesting may create a donor wound that does not heal properly. Grafting is associated with scarring, pain, and the risk of infection. Using allografts poses ethical considerations and entails problems with graft rejection.8 The scarcity of allogeneic skin and the expense of storage are further complicating factors. The problems associated with autogeneic, allogeneic, and xenogeneic skin grafts have prompted the search for an alternative that would be more widely available and would have properties as close to those of natural skin as possible.

This paper reviews the technical advances leading to the development of Apligraf(R) (Organogenesis Inc., Canton, Massachusetts), a tissue-engineered human skin product. Historical developments, animal modeling, manufacturing procedures, and clinical trials are reviewed for several evolving skin replacement products, along with their advantages and disadvantages. The mechanism of action of a human skin equivalent is discussed and its pharmacologic nature highlighted.

PIONEERING WORK IN TISSUE-ENGINEERED SKIN REPLACEMENT

Modern attempts to develop human skin replacements began in the early 1960s as a result of advances in tissue-- culture technology.9 The pivotal work of Rheinwald and Green10 at the Massachusetts Institute of Technology set the stage for subsequent tissue-engineering successes. These investigators overcame the limitations of propagating mammalian cells by cultivating human epidermal cells in serial culture, enabling the culture of these cells in large quantity.

Cultured (Expanded) Autologous Keratinocyte Grafts

Numerous investigators modified the Rheinwald-Green method to produce sheets of autologous keratinocytes suitable for grafting.3,11,12 A small biopsy sample of the patient's skin is subjected to trypsin disaggregation to separate the dermis from the epidermis. Isolated keratinocytes are serially subcultured and then expanded by in vitro procedures into sheets of epidermis over a period of 2 to 3 weeks. Lethally irradiated 3T3 cells may be used as a supporting fibroblast layer, and growth factors are added to the medium to prolong cell life span.11

Application of these cultured epithelial sheets to athymic mice has resulted in successful graft take in

most cases.13 O'Connor et al12 were the first to apply this technique clinically. They successfully treated two burn patients with cultured autologous expanded keratinocyte sheets. Compton et al13 demonstrated the appearance of histologic features of normal skin, including rete ridges and Langerhans' cells, after grafting of cultured keratinocytes. Adaptations of this procedure are now used to treat burns, venous ulcers, and other conditions requiring grafting.14 Success depends on many factors, including the type of surgical or chronic wound, the condition of the wound bed, the presence of bacterial contamination, the degree of pressure or friction, keratinocyte culture techniques, the patient's medical condition, and surgical and nursing techniques.15

The advantages of this method are that it is autologous, makes it possible to cover large areas with sheets from one small skin biopsy, results in permanent wound coverage with acceptable cosmetic results, leads to eventual regeneration of the dermis, and allows cryopreservation of cells for future use.16 There is a large body of clinical safety data on this procedure. Its principal drawback is that it requires a 3- to 4-week interval between biopsy and graft. Other disadvantages include the difficulty of handling the fragile keratinocyte sheets; the fact that the graft has only short-term stability, with only a 50% to 60% permanent take; and the lack of a dermal component, with neodermis formation often requiring years.5,16,17

Cultured Allogeneic Keratinocyte Grafts

Investigators began using allogeneic keratinocyte grafts primarily because of the long time required to obtain autologous sheets. As with any allogeneic transplantation, rejection was a particular concern. Rejection is thought to occur because of the presence of major histocompatibility antigens; however, as it turned out, this concern was mainly theoretical. Langerhans' cells in the epidermis play a major immunologic role. These cells, which originate from the monocyte cell line in the bone marrow and migrate to the basal layer of the skin, are the only cells in the epidermis that normally express human leukocyte antigens (HLA) capable of activating the immune system.4 Langerhans' cells are lost during serial culture of keratinocytes.18 Because these new keratinocyte cultures lacked Langerhans' cells, rendering them immunologically inert, they were expected to take and did take, without clinical evidence of graft rejection.19 It has since been found that donor cells do not persist in these allogeneic graft takes; rather, they are gradually replaced by recipient cells.20,21

For expanded allografts, neonatal foreskin is used to prepare the keratinocytes according to the techniques described by Green et al.11 When confluent cell monolayers are produced, they are transferred to gauze and either grafted or cryopreserved.22

The first successful application of allogeneic keratinocyte grafts in burn patients was reported in 1983.14 Subsequent experience has confirmed the acceptability of allogeneic keratinocyte grafts, with hundreds of patients benefiting from this procedure.23-25 Between 30% and 100% of these grafts take.1 Leigh et al26 reported on the use of allogeneic keratinocyte grafts in venous ulcers in 1987. Others27-29 have confirmed that in leg ulcers, while take was problematic, the allogeneic keratinocyte sheets promoted healing, often from the edges.

Cryopreservation makes it possible to stockpile keratinocytes, and mass cell culture renders them immediately available.30 Patients can be treated as outpatients without any need for biopsy. Allogeneic keratinocyte grafts act as a wound covering and promote healing. An important disadvantage is that epithelial sheets have no dermis. Wound contracture, instability and blistering of the graft, and inadequate cosmetic effects make the use of these cellular sheets less than ideal.

Autologous/Allogeneic Composites

The dermal component is important in skin grafting. The more dermis is grafted, the less wound contracture and scarring will occur and the more functional and rapid healing will be.3 Therefore, investigators have tried to develop skin replacements having a dermal component.

One method grafted cryopreserved allogeneic human cadaveric skin onto the wound. Several days later, the epidermis was removed, eliminating the most antigenic portion of the cadaveric skin. The exposed dermis was layered with either vacuum blister-prepared epidermis or cultured autologous keratinocytes. This composite was virtually nonantigenic and could be used with thin or widely meshed allografts.5 Animal and human trials of this method yielded acceptable but imperfect clinical results.6,23 Although the allogeneic dermis provided an extracellular matrix, it lacked the viable fibroblast component of the skin. There was inconsistent dermal vascularization, and the problem of keratinocyte fragility remained.

Acellular Collagen Matrices

Paralleling the development of composites was that of acellular matrices such as Alloderm(R) (LifeCell Corporation, The Woodlands, Texas) and Integra(R) (Integra Life Sciences Corporation, Plainsboro, New Jersey), which are used in the treatment of patients with full-thickness burns. Burke et al31 pioneered development of a bilayered artificial skin composed of a temporary Silastic(R) (Dow-- Corning Corporation, Midland, Michigan) "epidermis" bonded to a sponge of porous collagen (bovine)-chondroitin (shark) crosslinked with glutaraldehyde, which served as the dermis. This matrix produced successful wound closure in burn patients and took on many characteristics of human skin. After the graft became vascularized, the Silastic membrane was removed and replaced by epidermal grafts.

This type of matrix provided physical support and produced functional tissue at the graft site more rapidly than did epithelial sheets. The two-step application (matrix plus Silastic followed by epidermal grafts) was a disadvantage of this early skin matrix. In addition, the Silastic membrane inhibited drainage, increasing the risk of infection, hematoma formation, wrinkling with accumulation of serous exudate, and premature separation of the Silastic membrane.23 Other versions of acellular collagen matrices have incorporated autologous fibroblasts or cultured keratinocytes in the collagen matrix.32 In fact, dermal and epidermal cells were ultimately added to Integra.

Cellular Matrices

In improved versions of the collagen matrix, the Silastic membrane was replaced by cultured autologous human keratinocytes supported by a collagen-glycosaminoglycan (GAG) base that incorporated autologous fibroblasts.32 The open-matrix collagen framework allowed rapid ingrowth of fibrovascular tissue.33 Limited clinical studies in burn patients showed acceptable take, with formation of basement membrane within 9 days of graft placement32; approximately half of these grafts took.3 Unfortunately, the open-matrix collagen was highly susceptible to bacterial infection and enzymatic breakdown, which resulted in a 70% failure rate in several studies.6,23 The collagen-GAG matrix was degraded by increased collagenase activity, which, combined with the presence of microorganisms, led to graft failure.7 Even when antimicrobial agents were incorporated into the treatment regimen, grafts still failed. In separate studies using different manufacturing techniques, Boyce et al34 made cellular matrices of human epidermal cells and dermal fibroblasts added to bovine collagen and chondroitin-6-sulfate. These cultured skin equivalents stimulated healing in four chronic wounds.

Development of a Human Dermal Equivalent

Refinements of the matrix concept35 led to the development of Dermagraft(R) (Advanced Tissue Sciences, La Jolla, California), a living, metabolically active, immunologically inert dermal tissue.36 This tissue-engineered human dermis contains the normal dermal matrix proteins and cytokines. It consists of cultured neonatal fibroblasts grown within a polyglactin 910 or polyglycolic acid bioabsorbable mesh. (Polyglycolic acid has been used for bioabsorbable sutures and meshes for years.) In vitro growth allows the fibroblasts to proliferate and produce extracellular proteins.

The basic manufacturing steps are cell stock preparation, formation of matrix, and cellular ingrowth. In the first step, screened fibroblasts from human neonatal foreskin are enzymatically isolated and placed into tissue culture or banked. Second, after cell expansion, allogeneic dermal fibroblasts are placed onto a bioabsorbable polyglactin acid mesh (Vicryl(R), Ethicon, Inc., Somerville, New Jersey). Finally, the cell-seeded mesh is cultured for 2 to 3 weeks. The cells attach, spread, and proliferate within the mesh. As the fibroblasts proliferate, they secrete human dermal collagen, fibronectin, GAGs, growth factors, and other proteins. In effect, a dermal matrix is self-produced; no exogenous collagen is used.36

Recent research has shown that Dermagraft takes consistently in athymic mice.7 Preclinical and clinical trials indicate that the graft material is incorporated quickly into the wound and vascularizes well.36 The use of Dermagraft as a dermal substitute under meshed, split-thickness skin grafts led to complete wound closure within 14 days of take.37

Clinical trials36 in patients with diabetic foot ulcers have demonstrated more complete and rapid healing with Dermagraft than with conventional treatment. A dose response was evident, with patients who received a weekly application of Dermagraft for 8 weeks having the best healing rates. In this treatment group, 6 (50%) of 12 patients had complete healing, compared with 1 (8%) of 13 control patients ($P = 0.03$). Altogether, 11 (30%) of 37 Dermagraft-treated wounds closed completely, with no recurrences during an average of 14 months' follow-up.

The advantages of this living dermis include avoidance of nonhuman tissue, immediate graft application, mesh absorption in 60 to 90 days, and less wound contracture and scarring. Additional clinical trials are in progress to assess the efficacy of this product.

Dermagraft's precise mechanism of action has not been established. Gentzkow et al36 hypothesized that the fibroblasts produce matrix components and cytokines that stimulate healing. They suggested that the living fibroblasts respond physiologically to the recipient's tissue and modulate their secretion of growth factors.

Development of Apligraf

At the same time that Burke et al31 were developing matrices, Bell et al38 began work on the development of a human skin equivalent. Fibroblasts taken from human neonatal foreskin were cast in a bovine collagen lattice, which was seeded with epidermal cells to produce a bilayered skin equivalent. This organotypic model differed from previous attempts at developing a human skin replacement in that it contained both dermal and epidermal components, each housing living cells. Testing in animal models showed good graft take. This work was pivotal in the development of Apligraf(R) (also called Graftskin(TM)/Testskin(TM), Organogenesis Inc., Canton, Massachusetts).

Apligraf is a bovine collagen fibroblast-containing matrix integrated with a sheet of stratified human epithelium. The fibroblasts in the "dermis" and keratinocytes in the "epidermis" are viable, reproducing cells, making this one of the most advanced organ constructs to date.39 The process of manufacturing Apligraf is a modification of the procedure used by Bell et al38 and is described in detail by Parenteau et

al.39,40 The total manufacturing time is about 17 to 20 days. The basic steps are cell stock preparation, formation of lattice, epidermalization, stratification, and maturation. Fibroblasts and keratinocytes are first taken from screened neonatal foreskin and serially grown in tissue culture to establish cell banks.41 Next, a solution of purified acid-extracted bovine type I collagen is mixed with fibroblasts. This mixture is cast onto the porous membrane of the culture insert, where the neutralized mixture gels. The fibroblasts cause the collagen matrix to contract and form a dermal equivalent in approximately 4 to 6 days. In the epidermalization stage, keratinocytes are seeded onto the surface of the dermal equivalent and cultured for 2 days to allow coverage of the dermal equivalent. Cultures are then exposed to air to allow the epidermis to stratify. Finally, the material is cultured at the air-liquid interface to promote epidermal organization and maturation.

Apligraf is morphologically, biochemically, and metabolically similar to human skin.42 It has an organized morphology, typical proliferation kinetics, and lipid and keratin profiles characteristic of a morphologically and functionally differentiated epidermis.42 It appears to be immunologically inert, probably because of the absence of Langerhans' cells.43,44 The basement membrane contains stacks of lipid lamellae that lack the Landmann unit repeat, and the matrix contains short, banded collagen fibrils and extracellular matrix proteins, such as GAG, produced by the dermal fibroblasts.45,46 The dermoepidermal junction is flatter in Apligraf than in normal human skin, although the functional significance of this is unknown. The cell proliferation rate is similar to that of human skin.47 Mitotic activity occurs in the basal keratinocytes of the epidermis and in the fibroblasts within the matrix. After grafting onto nude mice, Apligraf continually adapts both physiologically and structurally to the wound site, coming to closely resemble intact skin.46 Hansbrough et al48 evaluated the properties of Graftskin in full-thickness wounds on athymic mice and found that it adhered quickly, with excellent take of all grafts. Vascular ingrowth occurred rapidly, and a structurally complete skin replacement was formed within 1 week of graft placement.

When used as a skin substitute for the in vitro testing of commercial products, Apligraf demonstrated properties similar to those of human skin.45 A recent report indicates that Apligraf can be made to take up genes introduced through retroviral vectors, allowing its use as a vehicle for gene therapy.49

Clinical Experience

Eaglstein et al50 evaluated Apligraf for treating wounds caused by the surgical removal of skin cancers or keratoacanthomas. The wound beds of 15 patients were grafted with Apligraf using absorbable sutures or no sutures. The graft took in 12 (80%) of the 15 patients. The healing course included phases in which Apligraf was translucent and/or fibrotic but pus-like in appearance. Wounds contracted 10% to 15%, slightly more than with full-thickness grafts. There was no toxicity or clinically detectable evidence of rejection, and there were no adverse events. Wounds were considered to be less painful and required fewer dressing changes and less attention than expected. Apligraf did not induce production of antibodies to bovine collagen. Moreover, Apligraf was easy to handle and improved healing. Additional clinical trials are under way in patients with nonhealing venous ulcers.

Frank clinical graft take is also observed with Apligraf, especially when used in venous ulcers.51 The dermal portion of Apligraf becomes vascularized within 14 days of placement.51 Apligraf rapidly changes the condition of and becomes integrated into the wound bed. It does not provoke inflammatory rejection reactions; however, the matrix is gradually replaced by natural remodeling of host cells that restores normal anatomic structure, function, and appearance to the site.51 Indeed, as with epithelial allografts, a human skin equivalent may be "silently rejected" by host cells and tissues. This process has also been termed "creeping replacement." Silent rejection may be immunologically based or may be a consequence of the product's containing too few or no stem cells, of slow cycling cells, or of some other process.

Results of a pivotal trial of Apligraf by Falanga et al51 are promising. In this trial, 293 patients with nonhealing venous ulcers were randomized to treatment with Apligraf or multilayered compression therapy. At 6-month followup, significantly more of the patients treated with Apligraf (63% vs 49%; P < 0.021) achieved complete wound closure and did so significantly faster (61 days vs 181 days; P <= 0.003) than did the patients receiving standard compression therapy.

A human skin equivalent may perform one or more functions in wound healing. First, it may serve as a biologic dressing that promotes healing by secondary intention. Second, the wound may heal by graft take, with remodeling of the tissue over time. Finally, a human skin equivalent may have pharmacologic action that stimulates healing.by recipient cells. Sabolinski et al52 consider Apligraf a "smart material" that can respond to its environment to achieve desired effects.

DISCUSSION

Originally, it was thought that expanded epidermal allografts were accepted by the body just as autologous skin grafts were, because human epidermal cells did not express HLA-DR antigens and because the histocompatibility antigens of the Langerhans' cells were lost. However, it has been discovered that with time, host cells replace the donor keratinocytes, meaning that cultured allografts ultimately do not survive.2

It is possible that even when the graft fails, significant healing takes place with biologically engineered skins because growth factors and cytokines stimulate rapid healing from the wound margins or appendage structures within the wound and stimulate production of granulation tissue within the wound bed. Factors that promote the cell regulation, growth, and differentiation found in keratinocyte supernates include interleukin 1, 3, 6, and 8; transforming growth factors alpha and beta; granulocyte-macrophage colony-stimulating factor; basic fibroblast growth factor; platelet-derived growth factor; tumor necrosis factor alpha; and many others.16,53 Because these growth factors and cytokines have been shown to have limited effects when applied alone to wounds, it is postulated that multiple factors act synergistically to promote epithelialization.14,17 It is also possible that tissue-engineered skin products provide or synthesize matrix components that allow more rapid reepithelialization.

Numerous investigators have shown stimulatory effects with various skin-grafting techniques.2,14,53 Chronic wounds (eg, those with dormant edges) reepithelialize when exposed to living allograft material. This edge effect is most probably due to the presence of stimulatory factors. Chronic wounds heal better after repeated application of skin grafts, suggesting that growth factors in the grafts are responsible. Kirsner et al54 demonstrated that prewounded autografts that were punched but left in place for 3 days to develop stimulatory processes produced better healing than did fresh autografts. In addition to more rapid healing at the wound bed, faster healing occurred at the prewounded donor sites than at the fresh donor sites. This initiation of the healing process before grafting demonstrates the pharmacologic role of skin grafts. Bioengineered tissues such as Apligraf and Dermagraft probably act as biologic systems for delivering growth factors to wounds.

CONCLUSIONS

The skin provides structure, form, and protection and performs regulatory functions. These functions are compromised when the skin is severely damaged, which can lead to substantial morbidity and mortality. Traditional grafting procedures, although effective, have many disadvantages. Availability is a major obstacle; hence, the search for a manufactured skin replacement has continued.

Ideally, a skin replacement should have the same properties as human skin.55,56 It should provide a permeable barrier to maintain body homeostasis, nitrogen balance, immune competency, and control of infection and should contain a supporting dermis to close the wound and prevent contraction and scarring. It should be easy to use, cosmetically acceptable, and cost-effective.57 New generations of tissue-engineered skin are working toward meeting these criteria.

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A comparison of methodologies for the preparation of human epidermal-dermal composites.

Ghosh MM, Boyce S, Layton C, Freedlander E, Mac Neil S.

Department of Plastic, Reconstructive and Burns Surgery, Northern General Hospital, Sheffield, UK.

The purpose of this study was to compare methodologies for the preparation of human skin composites based on deepidermized acellular dermal matrix, epidermal keratinocytes, and dermal fibroblasts with the aim of preparing a clinically useful skin substitute. Dermal matrices were prepared from normal human skin and we compared methods of sterilization (glycerol treatment, ethylene oxide treatment, and gamma irradiation), methods of removing the epidermis (sodium chloride, phosphate buffered saline, and dispase), and methods of seeding the composites with fibroblasts and keratinocytes. We report protocols for reproducibly preparing composites that share many of the features of normal skin after 7 days culture at an air-liquid interface. Such composites can be based on allodermis pretreated with either glycerol or ethylene oxide (although the latter gave less consistent results than the glycerol treatment). Fibroblast penetration into the dermis could be achieved by culture of cells on the reticular or papillary surface of the dermis. However, we report for the first time that fibroblast entry from the papillary surface only occurred when keratinocytes were also present.

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Transplantation of keratinocytes in the treatment of wounds.

Myers S, Navsaria H, Sanders R, Green C, Leigh I.

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BACKGROUND: Keratinocyte grafting can be used to treat acute traumatic and chronic non-healing wounds. The keratinocyte sheets are fragile and clinical "take" is difficult to assess, especially as activated keratinocytes secrete many growth factors, which have effects on wound healing apart from take. We have developed animal models of grafting that allow us to examine factors influencing autologous keratinocyte graft take. Results show clearly that pretreatment of the wound bed with viable dermis greatly increases the take of keratinocyte grafts. DATA SOURCES: International literature. CONCLUSIONS: As a greater understanding of the complex interactions of cell and matrix evolve, so will potential therapeutic maneuvers, not just in the field of cultured keratinocyte grafts, but clearly in that of benign tumors, for example, keloids, and that of oncology. There is now overwhelming evidence of the requirement for a dermal substitute for cultured keratinocyte autografts, and the sheet complexity of the situation demands that this should approximate live human dermis as closely as possible. The stumbling blocks relate to avoiding the risks of viral transmission, tissue matching of host and donor, providing early epithelial cover, and improving delivery systems for fragile keratinocyte grafts.

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Culturing skin in vitro for wound therapy.

Navsaria HA, Myers SR, Leigh IM, McKay IA.

Dept of Experimental Dermatology, London Hospital Medical School, UK.

Current tissue-culture techniques enable keratinocytes from a small piece of skin to be grown into sheets of epithelium, or cultured keratinocyte grafts, that are suitable for treating wounds. Serial subculture enables rapid expansion of a cell population, such that grafts of a total area equivalent to that of the surface of an adult can be obtained from an initial skin biopsy of approximately 2 cm2 in under one month. In this article, the methods currently used for culturing keratinocytes, the search for a fully functional replacement for the dermal elements of skin, and the prospects for clinical development of these technologies in the near future are discussed.

Publication Types:
- Review
- Review, tutorial

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